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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RE/PG4939A	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/03721	International filing date *(day/month/year)* 28.08.2003	Priority date *(day/month/year)* 30.08.2002
International Patent Classification (IPC) or both national classification and IPC A61K39/00		
Applicant GLAXO GROUP LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 32 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.03.2004	24.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Herrero, M Telephone No. +49 89 2399-8542

Form PCT/IPEA/409 (Cover Sheet) (January 2004)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/03721

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-6, 9-17, 19-32	as originally filed
7, 8, 18	received on 25.11.2003 with letter of 24.11.2003

Sequence listings part of the description, Pages

1-24	received on 15.12.2003 with letter of 12.12.2003

Claims, Numbers

1-7	as originally filed

Drawings, Sheets

1/11-3/11, 5/11, 9/11, 10/11	as originally filed
4/11, 6/11, 7/11, 8/11, 11/11	received on 25.11.2003 with letter of 24.11.2003

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations

see separate sheet

SECTION I

6. Additional observations

6.1 Sequence listing pages 1-24 filed with the letter of 12.12.03 do not form part of the application (Rule 13ter.1(f) PCT).

6.2 The amendments to the description and figures submitted with the Applicants' letter of 24.11.03 do not appear to contravene Art. 34(2)(b) PCT.

SECTION V

2. CITATIONS AND EXPLANATIONS

2.1 In view of the priority documents pertaining to the present application, the international patent application WO 02/070711 (publication date 12.09.02; filing date 01.03.02) cited in the International Search Report under the "P" category, is not to be regarded as state of the art according to Rule 64 (1) PCT, as the date of priority of 30.08.02 is validly claimed for the corresponding relevant parts of the present disclosure. This document (a co-pending patent application also referred to in the present description) is nevertheless brought to the Applicant's attention in view of the provisions of Article 54(3)(4) EPC.

2.2 The following documents have been considered for the purposes of this report:

D1: WO 00/65058 (also cited in the application)
D2: WO 01/62287 (also cited in the application)
D3: WO 01/34645
D4: WO 02/32450

2.3 Novelty and inventive step (Art. 33(2) and (3) PCT)

Having regard to the documents cited in the International Search Report the subject-matter hereby claimed (cf Claims 1-7) is considered to meet the novelty requirements of Art. 33(2) PCT, since a vaccine composition comprising the same components which should be contained in the hereby claimed vaccine

compositions appears not to be disclosed in the available prior art.

Nevertheless, the application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter claimed does not involve an inventive step (Rule 65(1)(2) PCT).

The technical problem underlying the present application may be regarded as the provision of an improved therapy for the treatment of diseases that are treatable with neutralization of interleukin 13 (IL-13), such as chronic obstructive pulmonary disease (COPD), asthma or atopic disorders such as atopic dermatitis or allergic rhinitis.

The present solution to the problem posed basically relies on autovaccine compositions based on modified autologous IL-13, capable of breaking the tolerance to self IL-13 antigen, which compositions comprise as adjuvant an immunostimulatory oligonucleotide containing at least one unmethylated CG motif and a saponin.

The hereby claimed solution appears not to involve an inventive step for the following reasons:

As outlined in the description (cf page 3, third paragraph) the state of the art provides evidence of the relevant role played by the Th2-type cytokine IL-13 in different pathological conditions, for instance, in the development of allergenic asthma (see also D2, page 4, lines 9-26 and the passage on page 2, lines 3-11 of D3).

The related prior art (e.g. D1 and D2) also reveals that the Th2-type cytokine IL-5 plays a prominent role in inflammatory responses accompanying conditions as asthma and other chronic allergic diseases.

In line with the above findings, both IL-5 and IL-13 molecules have been proposed in D2 as possible antigenic components for use in asthma or allergy vaccines, aimed at neutralisation of said cytokines (see e.g. Claim 7).

Moreover, D1 discloses a vaccine for the treatment of asthma, or other chronic allergic conditions, based on IL-5 analogues which are able to induce antibodies against self-IL-5. As noted in the paragraph bridging pages 4-5 of the present application, one of the types of modified IL-5 analogues employed for the vaccination purposes of D1 is an IL-5 immunogen, which has been supplemented with foreign T-cell epitopes whilst maintaining the IL-5 B cell epitopes. The methods employed to prepare said immunogenic IL-5 analogues, capable of breaking self-tolerance to autologous IL-5, are well established in the related technical field (see for instance the observations on page 4, 4th paragraph of the present application).

In view of the above, a skilled artisan facing the problem of providing an improved therapy for the treatment of asthma, devoid of the drawbacks associated with the current treatments mentioned on page 5, 2nd paragraph of the present disclosure, or alternative to the methods disclosed in D3 (see e.g. Claims 4 and 6), would be motivated to apply the teachings of D1 with equivalent effect to prepare an autologous vaccine based on IL-13.

On the other hand, for the vaccination purposes hereby pursued, the use of an adjuvant comprising a combination of a saponin and a immunostimulatory oligonucleotide containing at least one unmethylated CG motif merely represent one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill, in order to solve the problem posed. It is noted that at the date of priority of the present application the desirable properties of adjuvant compositions comprising oligonucleotides containing CpG motifs (e.g. the hereby referred to as OLIGO 4) and a saponin such as QS21 were well known, e.g. from D4, to the person skilled in the art (see also page 22, first paragraph of the present description).

Hence, no inventive merit is *a priori* recognisable in claiming the hereby proposed vaccine compositions (cf present Claims 1-7).

Furthermore, the experimental data provided in the present application (cf Examples 1 and 2 on pages 27-31) do not substantiate that a solution to the problem posed has been achieved. Example 1 relates to the preparation of a chimeric IL-13 molecule combining the sequence of predicted antigenic loops

derived from murine IL-13 with the sequence of predicted structural regions taken

from human IL-13. In Example 2 the required potency of an IL-13 autovaccine for treatment of asthma (in mice) is estimated by determining the level of mouse IL-13 neutralization required to overcome the "ovalbumin challenge" in the mouse asthma model, based on passive administration of rabbit anti-mouse IL-13 antibody (emphasis added). Finally, the "Vaccination studies" referred to in Example 3 only constitute an invitation to carry out pertinent tests whose results could eventually validate any inventive contribution associated with the hereby proposed therapeutic approach.

Accordingly the application as originally filed contains no suitable technical information which could substantiate the presence of any unexpected therapeutic result in connection, for instance, with the proposed methods of treatment described on page 22, last paragraph bridging over pages 23-24. These passages therefore appear to be of a mere predictive nature devoid of inventive merit vis-à-vis the teachings of the related prior art discussed above.

2.4 Further comments

(i) For the reasons outlined in the two last paragraphs of item 2.3 above, present Claims 1-7 are not (technically) supported by the description as required by Art. 6 PCT.

(ii) Claim 1 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. By merely referring to "an immunogen that is capable of generating an immune response in a vaccinee against self IL-13" the claim attempts to define the compositions of interest in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result.

The above deficiency (Art. 6 PCT) affects *mutatis mutandis* the subject-matter encompassed by Claims 2, 5 and 6 dependent thereon.

(iii) The intended vaccine composition according to instant Claim 7 appears to be redundant with the vaccine composition presently defined in Claim 4, contrary to the conciseness requirements of Art. 6 PCT.

(iv) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art

disclosed in the documents D3 and D4 is not mentioned in the description, nor are these documents identified therein.

situation (that is to say for use in vaccination of a human with a human protein sequence as the immunogen).

In one such embodiment of the present invention, the immunogens comprise a chimaeric IL-13 sequence that comprises substitution mutations to swap one or more of the human sequence amino acids with the equivalent amino acids found in the same positions within the sequence of IL-13 from another mammalian species. In the context of a human vaccine immunogen, the object of the chimaeric sequences is to maximise the amino acid sequence diversity between the immunogen and human native IL-13, whilst keeping maximal shape and conformational homology between the two compositions. The chimaeric immunogen achieves this by substituting amino acids found in regions predicted to be masked from the surface. Most preferably the amino acids are substituted with amino acids that are found in equivalent positions within an IL-13 sequence from another mammalian species. In this way, sequence diversity is achieved with minimal alteration to the overall shape/configuration of the immunogen.

In one aspect of the present invention, the human IL-13 immunogen comprises substitution mutations in areas that are associated with alpha helical regions, which substitutions involve swapping the human amino acid with the amino acid that appears in the same position within the IL-13 sequence of a different mammalian species.

Most preferably, there are substitution mutations in a plurality of sites within the IL-13 sequence, wherein at least two or more of the mutation sites comprise a substitution involving amino acids taken from different non-human mammalian species, more preferably the substitutions involve amino acids taken from 3 or more different non-human mammalian species, and most preferably the substitutions involve amino acids taken from 4 or more different non-human mammalian species.

Preferably, the substitutions in the human IL-13 sequence do not occur in at least six of the areas of high interspecies conservation: 3PVP, 12ELIEEL, 19NITQ, 28LCN, 32SMVWS, 50SL, 60AI, 64TQ, 87DTKIEVA, 99LL, 106LF.

The preferred IL-13 element of the vaccines of the present invention are human chimaeric IL-13 sequences which have a similar conformational shape to native human IL-13 whilst having sufficient amino acid sequence diversity to enhance its

immunogenicity when administered to a human, characterised in that the chimaeric IL-13 immunogen has the sequence of human IL-13 comprising:
(a) substitution mutations in at least two of the following alpha helical regions: PSTALRELIEELVNIT (SEQ ID NO. 24), MYCAALESLI (SEQ ID NO. 25), KTQRMLSGF (SEQ ID NO. 26) or AQFVKDLLLHLKKLFRE (SEQ ID NO. 27),
(b) comprises in unmutated form at least six of the following regions of high inter-species conservation 3PVP, 12ELIEEL, 19NITQ, 28LCN, 32SMVWS, 50SL, 60AI, 64TQ, 87DTKIEVA, 99LL, 106LF, and
(c) optionally comprises a mutation in any of the remaining amino acids,
wherein any substitution performed in steps a, b or c is a structurally conservative substitution.

The numerical prefix to the amino acids listed, refers to the positional number of the amino acid sequence in the mature form of human IL-13, wherein the first residue "G" is assigned the number 2.

In the context of step (a) of the above chimaeric IL-13 element, preferably at least two, more preferably at least three and most preferably all four alpha helical regions comprise at least one substitution mutation. In the context of step (b) preferably at least 7, more preferably at least 8, more preferably at least 9, more preferably at least 10, and most preferably all 11 of the regions are unmutated.

Preferably greater than 50% of these substitutions or mutations in the above chimaeric IL-13 element, comprise amino acids taken from equivalent positions within the IL-13 sequence of a non-human. More preferably more than 60, or 70, or 80 percent of the substitutions comprise amino acids taken from equivalent positions within the IL-13 sequence of a non-human mammal. Most preferably, each substitution or mutation comprise amino acids taken from equivalent positions within the IL-13 sequence of a non-human mammal.

Again in the context of the chimaeric human IL-13 element, preferably greater than 50% of these substitutions or mutations occur in regions of human IL-13 which are predicted to be alpha helical in configuration. More preferably more than 60, or 70, or 80 percent of the substitutions or mutations occur in regions of human IL-13 which are predicted to be alpha helical in configuration. Most preferably, each substitution or

preferred that the signal sequence is a non-human derived sequence that comprises a T-cell epitope, to further provide T-cell help. None of the disclosed preferred sequences have a stop codon as it may be useful to express them fused to other molecules eg immunoglobulin Fc, 6His to facilitate production or purification.

The numbering system used herein conforms with normal practice in the field of IL-13, in that the G in "GPVPP" is referred to as residue 2, and the remaining amino acids are numbered accordingly.

In one aspect of the present invention there is provided a method for the manufacture of a human chimaeric IL-13 vaccine comprising the following steps:
(a) taking the sequence of human IL-13 and performing at least one substitution mutation in at least two of the following alpha helical regions: PSTALRELIEELVNIT, MYCAALESLI, KTQRMLSGF or AQFVKDLLLHLKKLFRE,
(b) preserving at least six of the following regions of high inter-species conservation 3PVP, 12ELIEEL, 19NITQ, 28LCN, 32SMVWS, 50SL, 60AI, 64TQ, 87DTKIEVA, 99LL, 106LF,
(c) optionally mutating any of the remaining amino acids,
(d) attaching a source of T-cell epitopes that are foreign with respect to any human self epitope and also foreign with respect to any mammalian IL-13 sequence, to form an IL-13 immunogen, and
(e) combining the IL-13 immunogen with an adjuvant composition comprising a saponin and an immunostimulatory oligonucleotide comprising at least one unmethylated CG dinucleotide,
characterised in that any substitution performed in steps a, b or c is a structurally conservative substitution.

In the context of step (a) preferably at least two, more preferably at least three and most preferably all four alpha helical regions comprise at least one substitution mutation. In the context of step (b) preferably at least 7, more preferably at least 8, more preferably at least 9, more preferably at least 10, and most preferably all 11 of the regions are unmutated.

FIG. 5, Immunogen 1 (SEQ ID NO. 10)

```
5
          GGCCCTGTGCCTCCCTCTAGCGCCCTCAAGGAGCTCATTGAGGAGCTGGCCAACATCACC
        1 ---------+---------+---------+---------+---------+---------+   60
          CCGGGACACGGAGGGAGATCGCGGGAGTTCCTCGAGTAACTCCTCGACCGGTTGTAGTGG
          G  P  V  P  P  S  S  A  L  K  E  L  I  E  E  L  A  N  I  T
10
          CAGAACCAGAAGGCTCCGCTCTGCAATGGCAGCATGGTATGGAGCATCAACCTGACAGCT
       61 ---------+---------+---------+---------+---------+---------+  120
          GTCTTGGTCTTCCGAGGCGAGACGTTACCGTCGTACCATACCTCGTAGTTGGACTGTCGA
          Q  N  Q  K  A  P  L  C  N  G  S  M  V  W  S  I  N  L  T  A
15
          GGCATGTACTGTGCAGCCCTGGACTCCCTGATCAACGTGTCAGGCTGCAGTGCCATCGAG
      121 ---------+---------+---------+---------+---------+---------+  180
          CCGTACATGACACGTCGGGACCTGAGGGACTAGTTGCACAGTCCGACGTCACGGTAGCTC
          G  M  Y  C  A  A  L  D  S  L  I  N  V  S  G  C  S  A  I  E
20
          CGGACCCAGAGGATCTTGAGCGCCTTCTGCCCGCACAAGGTCTCAGCTGGGCAGTTTTCC
      181 ---------+---------+---------+---------+---------+---------+  240
          GCCTGGGTCTCCTAGAACTCGCGGAAGACGGGCGTGTTCCAGAGTCGACCCGTCAAAAGG
          R  T  Q  R  I  L  S  A  F  C  P  H  K  V  S  A  G  Q  F  S
25
          AGCTTGCGTGTCCGAGACACCAAAATCGAGGTGGCCCAGTTTGTAACGGACCTGCTCGTA
      241 ---------+---------+---------+---------+---------+---------+  300
          TCGAACGCACAGGCTCTGTGGTTTTAGCTCCACCGGGTCAAACATTGCCTGGACGAGCAT
          S  L  R  V  R  D  T  K  I  E  V  A  Q  F  V  T  D  L  L  V
30
          CATTTAAAGAGACTTTTCGCCAGGGAACGTTCAAC
      301 ---------+---------+---------+------  336
          GTAAATTTCTCTGAAAAAGCGGTCCCTTGCAAGTTG
          H  L  K  R  L  F  R  Q  G  T  F  N
35
```

FIG 11, Immunogen 7 (SEQ ID NO. 16)

```
TACGTACATTCCGACGGCTCTTATCCAAAAGACAAGTTTGAGAAAATCAATGGCACTTGG
---------+---------+---------+---------+---------+---------+
Y  V  H  S  D  G  S  Y  P  K  D  K  F  E  K  I  N  G  T  W

TACTACTTTGACAGTTCAGGCTATATGCTTGCAGACCGCTGGAGGAAGCACACAGACGGC
---------+---------+---------+---------+---------+---------+
Y  Y  F  D  S  S  G  Y  M  L  A  D  R  W  R  K  H  T  D  G

AACTGGTACTGGTTCGACAACTCAGGCGAAATGGCTACAGGCTGGAAGAAAATCGCTGAT
---------+---------+---------+---------+---------+---------+
N  W  Y  W  F  D  N  S  G  E  M  A  T  G  W  K  K  I  A  D

AAGTGGTACTATTTCAACGAAGAAGGTGCCATGAAGACAGGCTGGGTCAAGTACAAGGAC
---------+---------+---------+---------+---------+---------+
K  W  Y  Y  F  N  E  E  G  A  M  K  T  G  W  V  K  Y  K  D

ACTTGGTACTACTTAGACGCTAAAGAAGGCGCCATGCAATACATCAAGGCTAACTCTAAG
---------+---------+---------+---------+---------+---------+
T  W  Y  Y  L  D  A  K  E  G  A  M  Q  Y  I  K  A  N  S  K

TTCATTGGTATCACTGAAGGCGTCATGGTATCAAATGCCTTTATCCAGTCAGCGGACGGA
---------+---------+---------+---------+---------+---------+
F  I  G  I  T  E  G  V  M  V  S  N  A  F  I  Q  S  A  D  G

ACAGGCTGGTACTACCTCAAACCAGACGGAACACTGGCAGACAGGCCAGAAGGCCCTGTG
---------+---------+---------+---------+---------+---------+
T  G  W  Y  Y  L  K  P  D  G  T  L  A  D  R  P  E  G  P  V

CCTCCCTCTAGCGCCCTCAAGGAGCTCATTGAGGAGCTGGCCAACATCACCCAGAACCAG
---------+---------+---------+---------+---------+---------+
P  P  S  S  A  L  K  E  L  I  E  E  L  A  N  I  T  Q  N  Q

AAGGCTCCGCTCTGCAATGGCAGCATGGTATGGAGCATCAACCTGACAGCTGGCATGTAC
---------+---------+---------+---------+---------+---------+
K  A  P  L  C  N  G  S  M  V  W  S  I  N  L  T  A  G  M  Y

TGTGCAGCCCTGGACTCCCTGATCAACGTGTCAGGCTGCAGTGCCATCGAGCGGACCCAG
---------+---------+---------+---------+---------+---------+
C  A  A  L  D  S  L  I  N  V  S  G  C  S  A  I  E  R  T  Q

AGGATCTTGAGCGCCTTCTGCCCGCACAAGGTCTCAGCTGGGCAGTTTTCCAGCTTGCGT
---------+---------+---------+---------+---------+---------+
R  I  L  S  A  F  C  P  H  K  V  S  A  G  Q  F  S  S  L  R

GTCCGAGACACCAAAATCGAGGTGGCCCAGTTTGTAACGGACCTGCTCGTACATTTAAAG
---------+---------+---------+---------+---------+---------+
V  R  D  T  K  I  E  V  A  Q  F  V  T  D  L  L  V  H  L  K

AGACTTTTTCGCCAGGGAACGTTCAAC
---------+---------+-------
R  L  F  R  Q  G  T  F  N
```

FIG. 12, Immunogen 8 (SEQ ID NO. 17)

```
TCCTCTCATTCTTCTAACATGGCGAACACCCAGATGAAGTCCGATAAAATCATCATCGCG
---------+---------+---------+---------+---------+---------+
S  S  H  S  S  N  M  A  N  T  Q  M  K  S  D  K  I  I  I  A

CACAGGGGAGCTAGCGGGTATCTGCCTGAGCACACCCTGGAGTCCAAGGCTCTGGCGTTC
---------+---------+---------+---------+---------+---------+
H  R  G  A  S  G  Y  L  P  E  H  T  L  E  S  K  A  L  A  F

GCCCAGCAGGCTGACTACCTGGAGCAGGACCTGGCGATGACAAAGGATGGCCGCCTCGTG
---------+---------+---------+---------+---------+---------+
A  Q  Q  A  D  Y  L  E  Q  D  L  A  M  T  K  D  G  R  L  V

GTGATCCATGACCATTTTCTCGACGGACTGACCGACGTCGCCAAGAAGTTCCCCCACCGC
---------+---------+---------+---------+---------+---------+
V  I  H  D  H  F  L  D  G  L  T  D  V  A  K  K  F  P  H  R

CATAGGAAGGACGGGAGGTATTACGTGATTGACTTCACCCTCAAGGAGATCCAGAGCCTG
---------+---------+---------+---------+---------+---------+
H  R  K  D  G  R  Y  Y  V  I  D  F  T  L  K  E  I  Q  S  L

GAGATGACCGAGAACTTCGAGACCGGCCCTGTGCCTCCCTCTAGCGCCCTCAAGGAGCTC
---------+---------+---------+---------+---------+---------+
E  M  T  E  N  F  E  T  G  P  V  P  P  S  S  A  L  K  E  L

ATTGAGGAGCTGGCCAACATCACCCAGAACCAGAAGGCTCCGCTCTGCAATGGCAGCATG
---------+---------+---------+---------+---------+---------+
I  E  E  L  A  N  I  T  Q  N  Q  K  A  P  L  C  N  G  S  M

GTATGGAGCATCAACCTGACAGCTGGCATGTACTGTGCAGCCCTGGACTCCCTGATCAAC
---------+---------+---------+---------+---------+---------+
V  W  S  I  N  L  T  A  G  M  Y  C  A  A  L  D  S  L  I  N

GTGTCAGGCTGCAGTGCCATCGAGCGGACCCAGAGGATCTTGAGCGCCTTCTGCCCGCAC
---------+---------+---------+---------+---------+---------+
V  S  G  C  S  A  I  E  R  T  Q  R  I  L  S  A  F  C  P  H

AAGGTCTCAGCTGGGCAGTTTTCCAGCTTGCGTGTCCGAGACACCAAAATCGAGGTGGCC
---------+---------+---------+---------+---------+---------+
K  V  S  A  G  Q  F  S  S  L  R  V  R  D  T  K  I  E  V  A

CAGTTTGTAACGGACCTGCTCGTACATTTAAAGAGACTTTTTCGCCAGGGAACGTTCAAC
---------+---------+---------+---------+---------+---------+
Q  F  V  T  D  L  L  V  H  L  K  R  L  F  R  Q  G  T  F  N
```

FIG. 13, Immunogen 9 (SEQ ID NO. 18)

```
TTTAATAATTTTACCGTTAGCTTTTGGTTGCGTGTTCCTAAAGTATCTGCTAGTCATTTA
---------+---------+---------+---------+---------+---------+
F  N  N  F  T  V  S  F  W  L  R  V  P  K  V  S  A  S  H  L

GAAGGCCCTGTGCCTCCCTCTAGCGCCCTCAAGGAGCTCATTGAGGAGCTGGCCAACATC
---------+---------+---------+---------+---------+---------+
E  G  P  V  P  P  S  S  A  L  K  E  L  I  E  E  L  A  N  I

ACCCAGAACCAGAAGGCTCCGCTCTGCAATGGCAGCATGGTATGGAGCATCAACCTGACA
---------+---------+---------+---------+---------+---------+
T  Q  N  Q  K  A  P  L  C  N  G  S  M  V  W  S  I  N  L  T

GCTGGCATGTACTGTGCAGCCCTGGACTCCCTGATCAACGTGTCAGGCTGCAGTGCCATC
---------+---------+---------+---------+---------+---------+
A  G  M  Y  C  A  A  L  D  S  L  I  N  V  S  G  C  S  A  I

GAGCGGACCCAGAGGATCTTGAGCGCCTTCTGCCCGCACAAGGTCTCAGCTGGGCAGTTT
---------+---------+---------+---------+---------+---------+
E  R  T  Q  R  I  L  S  A  F  C  P  H  K  V  S  A  G  Q  F

TCCAGCTTGCGTGTCCGAGACACCAAAATCGAGGTGGCCCAGTTTGTAACGGACCTGCTC
---------+---------+---------+---------+---------+---------+
S  S  L  R  V  R  D  T  K  I  E  V  A  Q  F  V  T  D  L  L

GTACATTTAAAGAGACTTTTTCGCCAGGGAACGTTCAAC
---------+---------+---------+---------
V  H  L  K  R  L  F  R  Q  G  T  F  N
```

Figure 18, SEQ ID NO. 23


```
                GGGCCGGTGCCAAGATCTGTGTCTCTCCCTCTGACCCTTAGGGAGCTCATTGAGGAGCTG
      1         ---------+---------+---------+---------+---------+---------+      60
                G  P  V  P  R  S  V  S  L  P  L  T  L  R  E  L  I  E  E  L

                GTCAACATCACACAAGACCAGACTCCCCTGTGCAACGGCAGCATGGTATGGAGTGTGGAC
     61         ---------+---------+---------+---------+---------+---------+     120
                V  N  I  T  Q  D  Q  T  P  L  C  N  G  S  M  V  W  S  V  D

                CTGGCCGCTGGCGGGTACTGTGCAGCCCTGGAATCCCTGACCAACATCTCCAATTGCAAT
    121         ---------+---------+---------+---------+---------+---------+    180
                L  A  A  G  G  Y  C  A  A  L  E  S  L  T  N  I  S  N  C  N

                GCCATCGAGAAGACCCAGAGGATGCTGGGCGGACTCTGTAACCGCAAGGCCCCCACTACG
    181         ---------+---------+---------+---------+---------+---------+    240
                A  I  E  K  T  Q  R  M  L  G  G  L  C  N  R  K  A  P  T  T

                GTCTCCAGCCTCCCCGATACCAAAATCGAGGTGGCCCAGTTTGTAAAGGACCTGCTCAGC
    241         ---------+---------+---------+---------+---------+---------+    300
                V  S  S  L  P  D  T  K  I  E  V  A  Q  F  V  K  D  L  L  S

                TACACAAAGCAACTGTTTCGCCACGGCCCCTTCTAA
    301         ---------+---------+---------+------  336
                Y  T  K  Q  L  F  R  H  G  P  F  *
```